B1, con't

D. close-packing the resulting porous particles to form said matrix.

Delete claim 21.

B2

~~30~~ 10. (Amended) The method of claim ~~20~~ 1 comprising the additional step of adsorbing osteogenic protein onto said particles prior to step D.

REMARKS

Reconsideration of this application is requested. Independent claim 20 and dependent claims 22-34 are now in this application.

Claim 20 has been amended to more particularly describe the nature of the process of this invention, and to distinguish it further over the prior art. Support for these amendments can be found, for example, on page 10, line 28 through page 11, lines 1-6 and lines 12-14; page 12, lines 13-18; page 25, lines 1-4; page 32, lines 21-24, and throughout the Specification. Claim 21 has been deleted as the amendments to claim 20 render this dependent claim redundant.

J6

The Specification has been amended to correct minor typographical errors.

At the outset, the undersigned attorney wishes to thank Examiner Nutter for his time and consideration during the interview which took place at the Patent Office on June 7, 1990. The substance of the interview is described in the Examiner Interview Summary Record.

Applicants' invention is directed to a method of making a biocompatible bone particle matrix suitable for implantation cross-species into a mammalian host, including humans. To manufacture the matrix, Applicants start with fine particles obtained from xenogenic bone that are demineralized and guanidine-extracted. The particles are insoluble and comprise Type 1 bone collagen. Known particles of the type disclosed in some of the cited references may be used. The novel feature of this invention, and the feature which allows the matrices to be implanted xenogenically, is the treatment of the particles with a "swelling" or "fibril modifying" agent <u>to increase the particle porosity and intraparticle surface area</u> while maintaining the particulate nature of the extracted, deminearalized bone powder. After washing, the resulting porous particles are biodegradable in the host, act as a substrate for cellular ingrowth without requiring chemical

crosslinking, do not induce unacceptable inflamation, and may be used xenogenically, i.e., cross-species.

The increased porosity and surface area resulting from the treatments disclosed in the present application are evident from a comparison of the scanning electron micrographs in Figures 1A through 2E of the Drawings, which provide photographic evidence of the effect of the treatment on the overall architecture of the matrix particles. In addition, analyses by the Applicants since the filing of this Application (and disclosed in U.S. 483,913, a continuation-in-part of the present application), conducted on matrix material produced as disclosed herein, verify that treatment of the particles with swelling or fibril modifying agents at least doubles the particle surface area, and increases its intrusion volume by at least 25% and often more than 50% over untreated particles. The detailed chemical and physical analysis further has indicated that treatment with swelling agents increases the number and size of micropits on the particle surfaces. The claimed process thus starts with insoluble, quanidine-extracted particles which are treated <u>as particles</u> and never broken down by way of dissolution during the process. The claims have been amended to describe more particularly these novel features of the invention.

The claims presently stand rejected under 35 USC §103 as being unpatentable over Wang (WO88/00205), Jeffries (US 3,394,370), and Sampath (PNAS vol.80), in view of Thiele et al. (US 4,172,128). The Examiner states that all the steps used in the production of the matrices of this invention are shown by the prior art and subsequent use of them to produce the matrix of this invention "would have been obvious to a practitioner having ordinary skill in the art at the time the invention was made, in the absence of any showing of any unexpected results." Applicants respectfully traverse this rejection to the extent it is applied to the claims as amended.

The applied Sampath reference describes a method for preparing allogenic bone matrix. That is, a method for preparing bone matrix from a given species for implantation in that same species. The method is well known in the art and is described in an earlier paper by the same authors (PNAS, 78:7599-7603, (1981)). The method involves demineralization of pulverized allogenic bone matrix, followed by multiple dissociative extractions of the matrix with 4M guanidine hydrochloride (Gdn-HCl) to remove non-collagenous proteins associated with the bone collagen, including any osteoinductive factors. This treatment of bone collagen renders the material suitable as a carrier material for osteoinductive factors to be implanted in an allogenic host, (e.g., implants of rat bone

matrix in rats, or rabbit bone matrix into rabbits.) In fact, this is also the method used in the applied Wang reference for their allogenic implants (see p. 15, last line, through p.16, line 1). However, the treatment is not successful for the preparation of xenogenic matrices, (for example, bovine matrix into rat or rabbit). Those skilled in the art desiring to use a carrier material comprising bone collagen particles therefore have been limited to using allogenic matrices, a process that is both expensive, tedious, and generally economically unrealistic for human applications. Following Applicants' method it is now possible, using the material disclosed in the Wang or Sampath references, to prepare useful <u>xenogenic</u> carriers. Applicants respectfully submit that nothing in the applied Wang or Sampath references suggests or teaches how to modify the allogenic bone matrix material disclosed in those references to obtain the xenogenically biocompatible porous material of this invention, nor suggest that such conversion is possible.

An apparent alternative to matrices derived from bone particles is the matrix preparation disclosed in the applied Jeffries reference comprising purified, reconstituted Type I collagen. Reconstituted collagen is collagen that has been highly purified (> 99%) <u>by dissolving the collagen fibers</u>, removing associated non-collagenous components, and then <u>allowing the collagen to renature</u> into its fibrillar form.

During purification, the potentially immunogenic telopeptides (also the primary source of interfibril crosslinks) are removed enzymatically. Jeffries discloses a matrix said to be suitable for xenogenic implants formed by dispersing reconstituted collagen in acetic acid to form a disordered matrix of elementary collagen molecules that is then mixed with an osteogenic factor and lyophilized to form a "semi-rigid foam or sponge."

In contrast to the matrix of Applicants' invention, the matrix disclosed by Jeffries does not comprise bone particles. Moreover, the disordered collagen, substantially reduced in fibril structure, has little structural strength. Such matrices can be provided with additional structural stability by chemical crosslinking (the preferred embodiment of the Jeffries invention). However, chemical crosslinking agents, such as glutaraldehyde (the crosslinking agent suggested by Jeffries) may have adverse biological effects, such as cell cytotoxicity (see Cooke, et al. (1983) British J Exp Path, 64:172). It should be noted that Jeffries does not present any implant data using these matrices. In any event, the Jeffries matrix is very different from the matrix of Applicant's invention.

As the Examiner points out, Jeffries discloses using his dispersed purified collagen matrix together with demineralized bone particles. However, the bone particles are used as a source of osteogenic activity only (they are not extracted with guanidine hydrochloride), and not as a carrier material (compare Examples 1 and 2). Moreover, the bone particles used are allogenic (see Example 1, col.3). Applicants respectfully submit that nothing in Jeffries suggests or teaches how to modify demineralized bone particles to obtain the xenogenically biocompatible, porous matrix particles of this invention.

The Examiner has applied the Thiele reference for its teaching of treating demineralized bone particles with swelling agents. The Examiner states that the Thiele reference is relied on to "show the swelling step for enhanced adsorption of other agents."

Thiele discloses a method of producing bone-like material of reduced immunogenicity, said to be useful for implantation cross-species. The process involves treating demineralized bone particles with swelling agents to swell the collagen and to dissolve the mucopolysaccharides (also known as glycosaminoglycans) associated with the collagen. The now dissociated mucopolysaccharides then are removed and the swollen collagen fibers are dissolved in 2N sodium hydroxide to

form a homogeneous colloidal solution or sol comprising collagen in its elementary form. Once the original bone material has been thus completely degraded, collagen fibers are regenerated by adding physiologically inert mucopolysaccharides to the sol and exposing this solution, in a mold, to an electrolyte to form a solidifed gel comprising oriented fibrils and fibril bundles with "straight, parallel capillaries...extending vertically to the filamentary molecules" (col.4, lines 12-15). In addition, as indicated in col. 6, lines 33-36, reorganization of the collagen into its "natural" form causes shrinking of the sol "due to the compaction of the tightly arranged and oriented collagen fibrils and to their partial dehydration". The gel then can be chemically crosslinked and/or remineralized.

The use of swelling agents or "fibril modifying" agents in Applicants' invention has a different purpose and a different effect than that of the Thiele reference. The collagen swelling step in Thiele serves only to remove unwanted non-collagenous components, namely mucopolysaccharides. This use of swelling agents to purify collagen fibrils is well known in the art (see, for example, US 3,520,402, to Nichols et al.). The purified, swollen collagen fibers are then dissolved and reconstituted. By contrast, it is the series of the guanidine extraction steps which may be conducted to produce

the starting material for the method of Applicants' invention that removes unwanted non-collagenous components. The swelling treatment of Applicants' matrix serves primarily to modify the <u>architecture</u> of the bone particles, by increasing the porosity and surface area of the particles, without dissolving the particle. The matrix of Applicants' invention remains a composite of discrete porous particles, as compared with the gel of reconstituted fibers of the Thiele reference. Applicants respectfully submit that nothing in the Thiele reference teaches or suggests using swelling agents to alter the overall architecture of the bone particles in their starting material, or the desirability of this effect, in order to form the porous matrix particles of Applicants' invention.

35 USC §103 states the the subject matter, taken as a whole, must be considered when evaluating the patentability of an invention under §103. The case law on this is quite clear. Applicants submit that, although some steps in their method can be found in the applied references, the combination of the references does not teach or suggest the subject matter taken as a whole. The references, fairly interpreted collectively do not teach Applicant's method of forming biodegradable matrices comprising demineralized, guanidine-extracted, xenogenically compatible particles of increased porosity and surface area.

Applicants contend that their invention is distinct from and unobvious over Wang, Sampath, and Jeffries, taken with Thiele, and therefore is free of the prior art.

On the basis of the above amendments and remarks, reconsideration and allowance of the Application and the claims is requested.

Respectfully submitted,

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